Thermodynamic and Structural Studies on the Human Serum Albumin in the Presence of a Polyoxometalate

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The interaction of a polyoxometal (POM), $K_6SiW_{11}Co(H_2O)O_{39}.10H_2O$ (K_6) as a Keggin, with human serum albumin (HSA) was studied by different methods and techniques. Binding studies show two sets of binding sites for interaction of POM to HSA. Binding analysis and isothermal calorimetery revealed that, the first set of binding site has lower number of bound ligand per mole of protein (ν), lower Hill constant (n), higher binding constant (K), more negative entropy (ΔS) and more electrostatic interaction in comparison to the second set of binding site. In addition, differential scanning calorimetery (DSC) and spectrophotometery data showed that, there are two energetic domains. The first domain is less stable (lower T_m and C_p) which corresponds to the tail segment of HSA and another with more stability is related to the head segment of HSA. Polyoxometal also decreases the stability of protein as T_m, secondary and tertiary structure as well as quenching of the fluorescence decrease. On other hand, perturbations in tertiary structure are more than secondary structure.

Key Words : Polyoxometalate, Human serum albumin, Isothermal titration calorimetery, Circular dichroism, Fluorescence

Introduction

Albumin is a major plasma protein and binds to the number of drugs altering their pharmacokinetics. It is single chain polypeptide of 585 residues, which comprises about 60% of the plasma protein. It is the major contributor to the oncotic of blood.[1] In addition, it has been reported that albumin is chiefly responsible for maintainance of blood pH.[2,3] The human serum albumin (HSA) is named a multi-functional plasma carrier protein because of its ability to bind to an unusually broad spectrum of ligands. These includes inorganic cations, organic anions, various drugs, amino acids, and perhaps most important and physiologically available hydrophobic molecules such as bilirubin and fatty acids.[1,4,5]

In mammals, albumin is synthesized by the liver and possesses a half-life in circulation of 19 days.[6,7] Serum albumin has been one of the most studied proteins for 40 years, because its primary structure is very well known for a long time and its tertiary structure was determined a few years ago by X-ray crystallography.[5,6] Its primary structure is characterized by low content of tryptophan, a high content of cystein stabilizing a series of main loops, and charged amino acids such as aspartic and glutamic acids, lysine and arginine.[5] The apparent repeating structural features, large and small loops and connecting segments, suggest that albumin evolved through a series of gene duplications.[5] Its secondary structure is constituted of 67% of helix of six turns and 17 disulfide bridge.[5,8] The tertiary structure is composed by three domains I, II, III. Each domain is constituted by a cylinder formed by six helices, and each one of these domains are constituted by two subdomains formed by three helices that covalently linked by their double Cys bridge. These subdomains are IAB, IC, IIAB, IIC, IIIAB, IIIC.[5,9] The binding cavity in the domain IIIA is known to be able to bind many ligands like, for example, digitoxin, ibuprofen and tryptophan. Aspirin and iodinated aspirin derivatives show nearly equal distributions between the binding sites located in subdomains IIA and IIIA. Warfarin occupies a single site in IIA.[6,8] Many small aromatic carboxylic acids are equally distributed in both IIA and IIIA.[6] Since domains II and III share a common interface. It is known that binding to domain III leads to conformational changes affecting the binding affinities to domain II. Trp214 residue plays an important structural role in the formation of the IIA binding site by limiting the solvent accessibility and besides that it participates in additional hydrophobic packing interactions at IIA-IIIA interface.[7] Cys 34 used as a fluorescent probe to monitor the surroundings of this residue.[10-12]

On the other hand, same as all of ligands, several compounds such as polysulfates, polysulfunates, polycarboxylates, polyphosphates and polyoxometalates (POM) have been identified that pharmaceutical effect and inhibit an early stage in the replicative cycle of the human immunodeficiency virus (HIV).[13] Polyoxometalates are early transition metal oxygen anion clusters. More specifically, they are oligomeric aggregates of metal cations (usually the d^o species V^V, Nb^V, Ta^V, Mo^{VI}, and W^{VI} bridged by oxide anions that are formed by self-assembly processes. There are two generic families of POMs, the isopoly and the heteropoly